Degradation of Organophosphorous Nerve Agents by Enzyme-Polymer Nanocomposites: Efficient Biocatalytic Materials for Personal Protection and Large-Scale Detoxification

Iqbal Gill, Antonio Ballesteros

Department of Biocatalysis, CSIC Institute of Catalysis, Campus Universidad Autonoma, 28049 Madrid, Spain

Received 25 December 1999; accepted 13 July 2000

Abstract: The biocatalytic destruction of organophosphates has become an important focus area, as efficient "clean" technologies are sought for chemical weapons decommissioning, counteracting nerve agent attacks, and protecting against organophosphate pesticide poisoning. A novel method is advanced for immobilizing the broad-spectrum enzyme organophosphorous hydrolase (OPH) from *Pseudomonas diminuta*, based on the formation of nanocomposite protein-silicone polymers. The resulting materials are highly active, stable, and versatile biocatalysts for the liquid and gas phase detoxification of organophosphates, and can be fabricated as monoliths, sheets, thick films, granulates, or macroporous foams. This approach offers an efficient avenue to robust, high-performance biocatalytic OPH-containing polymers that outperform immobilized OPH catalysts reported to date. The method provides for the first time a route to biocatalytic materials that may be suitable for "active" protective wear, as well as bulk catalysts for the destruction of large volumes of organophosphates. The preparation of OPH-silicone biocomposites, their performances in the liquid and gas phase detoxification of paraoxon, dichlorvos, and diisopropyl fluorophosphate, and their features are discussed. © 2000 John Wiley & Sons, Inc. *Biotechnol Bioeng* 70: 400–410, 2000.

Keywords: organophosphorous hydrolase; immobilization; silicone composites; nerve agent; detoxification

INTRODUCTION

Organophosphorous nerve agents (OPs) are a major issue worldwide, most visibly in the chemical weapons arena in relation to the decommissioning of arms stockpiles and the protection of personnel from warfare agents (Brin, 1993; Holm, 1996; NRC Report, 1993). World stockpiles of nerve agents exceed 200 kilotons, with US reserves alone amounting to over 30 kilotons, a scenario that poses a formidable challenge to disarmament. Also, recent events have seen mass military and civilian casualties resulting from the wartime deployment of OP weapons in the Middle East and the terrorist use of sarin in Japan. In addition, organophosphorous pesticides are widely utilized in animal and crop protection, and worldwide over 0.7 million cases of poisoning are believed to result from occupational and incidental exposure to OPs (Minton and Murray, 1988).

The hazardous nature of OPs and their wide usage has led to concerted efforts to develop methods for the destruction of these toxins (Grimsley et al., 1997; Holm, 1992; NRC Report, 1993). Conventional approaches of incineration and chemical hydrolysis/oxidation (Holm, 1992; NRC Report, 1993) are hindered by their high environmental impact and logistics and operational safety issues. Similarly, personal protection has relied on passive technologies, namely, OP adsorption by activated carbons, resulting in unwieldy protective wear of limited protective capacity. In an effort to find safer and more efficient alternatives, attention has turned to "clean" technologies (Grimsley et al., 1997; NRC Report, 1993), especially biotechnological methods based on the organophosphorous hydrolase enzyme from *Pseudomonas diminuta* (OPH; EC 3.1.8.1), which can hydrolyze a range of OPs containing P–O, P–S, P–CN, and P–F bonds (Benning et al., 1994; Dumas et al., 1989; Hong and Raushel, 1996; Hoskin et al., 1989; Kolakowski et al., 1997; Lai et al., 1995; Landis and DeFrank, 1991; Rastogi et al., 1997).

Practical implementation, sustained performance, and economics would in many cases necessitate OPH-based detoxification technologies to utilize immobilized biocatalysts. Weapons decommissioning demands physicochemically robust catalysts which are stable in long-term use and which can cope with high throughputs of concentrated OP feeds. The prerequisites for protective wear are more critical—lightweight "active" fabrics and filter elements which rapidly and completely detoxify liquid-phase and gaseous OPs agents upon contact are essential, and the materials should be operable for extended periods under various climates and be reusable.

Correspondence to: I. Gill
Present address for authors: Biotransformation and Biocatalysis Section, Biotechnology Center of Excellence, Roche Vitamins Inc., 340 Kingsland Street, Nutley, NJ 07110; telephone: +01-973-284-6132; fax: +01-973-284-5979; e-mail: iqbal_s.gill@roche.com or iqbalsgill@hotmail.com

OPH immobilized within/on polymers that are amenable to mass fabrication would appear to offer the best solution. Although OPH has been immobilized on nylon, polyurethanes, and photopolymerized PEG-based hydrogels and sol-gel silicates (Andreopoulos et al., 1999; Caldwell and Raushel, 1991; Gill and Ballesteros, 1998; Havens and Rase, 1993; Le Jeune et al., 1997), fabrication/processing and operational limitations have hindered applications. Thus, OPH-polyurethane foams are excellent as clean-up sponges, but their use in protective wear has been less successful (Caldwell and Raushel, 1991; Havens and Rase, 1993).

Herein, we present a new class of immobilized OPH catalysts, namely, nanocomposites formed by incorporating OPH into silicone polymers. The protein-silicone hybrids are highly active, stable under long-term storage and operation, and can be fabricated and employed in a variety of physical forms for detoxifying liquid and gas-phase OPs. The method provides a versatile avenue for fabricating OP-degrading biocatalytic sheets, thick films, granulates, and solid foams for protective wear and high-volume destruction applications.

MATERIALS AND METHODS

Materials

Fumed silica (14 nm), δ-gluconolactone, tetramethyl orthosilicate, Tweens, triethanolamine, PVA, glycerol tris[poly(propylene glycol)], diazabicyclooctane (DABCO), nitrile gloving, and all solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI). HEPES, TRIS, DFP, and paraoxon were obtained from Sigma Chemical Co. (St. Louis, MO). Hypol prepolymers were obtained from Hampshire Chemical Corp. (Lexington, MA). Silanol-terminated poly(dimethylsiloxane) (ST-PDMS), poly(hydrogenmethylsiloxane), poly(diethyl silicate) (PDES), 3-aminopropyltriethoxysilane, zinc(II) 2-ethylhexanoate, and tin(II) 2-ethylhexanoate (TO) were obtained from ABCR GmbH (Karlsruhe, Germany). Co(II)-OPH (4.57 kU mg^{-1}, for paraoxon hydrolysis) was prepared from *Pseudomonas diminuta* according to published procedures (Benning et al., 1994).

Analytical Techniques

HPLC was carried out using a Waters 510 pump system connected to a Waters (Rochester, MN) 717 Autosampler, a Waters II Diode Array detector, and a Waters data collection station, or on an LDC Milton Roy CM4000 pump connected to a Spectra Physics (Mountain View, CA) SP8450 UV/VIS detector, LDC Marathon autosampler, and an HP 35900 Chemstation. Analyses were performed with a Hichrom RPB5 column (5 μm, 0.46 × 15 cm) eluted with water-methanol, 30°C or a Hypercarb column (10 μm, 0.46 × 10 cm) eluted with water-acetonitrile, 30°C. GC was performed on a Varian (San Fernando, CA) Star 3400CX capillary GC equipped with an FID detector, and connected to a Varian Star data acquisition station. Analyses were performed on a 30 m SPB-5 column (0.50 mm diameter, 0.5 μm film thickness) with helium as carrier gas. Analyses were carried out using an injection temperature of 250°C, a split ratio of 25–100, a total flow of 35–40 mL/min, a detector temperature of 250°C, and a ramp of 50–250°C over 20–30 min. UV-VIS spectrometry was carried out on a Perkin Elmer Lambda-2 spectrometer equipped with a thermostatted, stirred cell, or a Shimadzu UV-1602 spectrometer.

Preparation of Isocyanatopropyl-Silica

A suspension of silica (100 g of 14 nm silica) in hydrochloric acid (500 mL of 0.2 M in 5:1 water-ethanol) was heated to 70°C for 5 h, centrifuged (15,000*g*, 10 min), then dried under nitrogen. The product (50 g in 800 mL cyclohexane) was shaken with 3-isocyanatopropyltriethoxysilane (50–100 mmol in 200 mL cyclohexane) and 3 Å molecular sieves (20 g) at 20°C for 20 h, the mixture filtered through a coarse sieve, then rotary evaporated to dryness at 20°C. This furnished 3-isocyanatopropyl-silica with 0.93–1.88 mmol isocyanate g^{-1} (as determined by titration with benzylamine).

Preparation of Poly(hydroxymethylsiloxane) (PHOMS)

Poly(hydrogenmethylsiloxane) (500 g in 500 mL acetone) was added over 2 h at RT to a stirred mixture of zinc chloride (20 g), zinc 2-ethylhexanoate (10 g), and acetone (3 L). After 20 h the solution was concentrated at 20°C, diluted with 3:1 isopropanol-water (1.5 L), hydrolyzed with sodium hydroxide (2.3 L, 5.0 M aqueous, RT, 2 h), and the silanolate decomposed with HCl (5.0 M aqueous, to pH 4–6, over 1 h). The suspension was filtered, washed with 9:1 water-isopropanol (6 × 1 L) and the PHOMS stored wet (55–60% w/w solids, 8.8–10.7 mmol silanol g^{-1}).

OPH-Silica Adsorbates

Isocyanatopropyl-silica (1 g in 10 mL of 0.1 M ice-cold TRIS, pH 7.5) was shaken with OPH enzyme solution (0.2–2 mL of 2.29 kU mL^{-1} or 0.2–10 mL of 22.9 kU mL^{-1}, in 0.1 M TRIS, pH 7.5, with 1 mM $CoCl_2$) at 5°C for 10 h. The suspension was centrifuged (15,000*g*, 10 min, 5°C), the solid resuspended in 10 mL of buffer containing 10 mM ethanolamine, centrifuged, and the procedure repeated. The wet solid was dried under nitrogen at 5°C and stored at −15°C. Unbound OPH in the supernatants was determined by Lowry protein and paraoxon hydrolysis assays.

OPH-PHOMS Adsorbates

PHOMS (2 g in 15 mL of 0.1 M ice-cold TRIS, pH 7.5 with 10% v/v isopropanol) was stirred with OPH solution (0.4–10 mL, 22.9 kU mL^{-1}, in 0.1 M TRIS, pH 7.5, with 5% v/v isopropanol and 1 mM $CoCl_2$) at 5°C for 10 min, then

phosphate buffer (15–20 mL, 0.5 M, pH 7.5, with 1 mM $CoCl_2$) added, and stirring continued for 3 h. The suspension was filtered, the gel washed with ice-cold buffer, ice-cold acetone, then ice-cold pentane (3 × 5 mL of each), and the cake dried under nitrogen at 5°C, then stored at −15°C.

Monolithic OPH-Silica-Silicone Composites

OPH-silica (3.5–7.2 g) was suspended in ST-PDMS (9.2–12.0 g of a mixture containing 30–70% w/w of 0.1% functional and 70–30% w/w of 1.1% w/w functional prepolymers), the suspension blended with a mixture of PDES (2.5–4.0 g), PAPS (0.4–3.0 g) and TO (40–100 mg), and the mix poured into a 5 × 5 × 1 cm aluminum foil mold. The silicone was cured in air at room temperature for 5–10 h to provide a rubber with a density of 1.08–1.34 g mL^{-1}. This was granulated, either in the dry state using a Centrifugal Grinding Mill (Retsch ZM100 Mill, cooled with dry ice), or suspended in cyclohexane (cooled in dry ice-acetone) and wet-processed with a homogenizer (Polytron PT3100). The granulate (0.15–3 mm) was washed with cyclohexane, then dried in air for 5 h. The material (5–20 g) was hydrophilized by reaction with δ-gluconolactone (100–300 mL , 10% w/v in 0.1 M TRIS, pH 7.5, containing 15–20% v/v propan-2-ol and 2 mM $CoCl_2$) at 5°C for 1–5 h, then filtered and washed with buffer (2 × 100 mL of 0.1 M TRIS, pH 7.5, with 10% v/v propan-2-ol and 1 mM $CoCl_2$, then 2 × 50 mL of pure buffer), and stored wet at 5°C, or dried and stored at −15°C.

Thick Film OPH-Silica-Silicone Composites

OPH-silica (0.9–1.3 g) was dispersed in ST-PDMS (4.5–5.2 g of a mixture containing 70–100% w/w of 0.1% w/w functional and 30–0% w/w of 1.1% w/w functional prepolymers), this combined with a mixture of PDES (0.7–2.0 g), poly(3-aminopropylethoxysiloxane) (PAPS 0.8–2.0 g), and TO (20–50 mg), and the blend cast onto the substrate (primed with 3-aminopropyltriethoxysilane) using a 10 mm diameter Teflon® spreading rod, and 180, 280, and 360 μm spacer templates constructed from 3M Correction Tape (90 μm). Curing under ambient conditions for 8–20 h provided a flexible thick film (150–270 μm) that adhered strongly to the underlying substrate. This was washed with cyclohexane, dried in air at room temperature, then hydrophilized as above.

Foamed OPH-Silica-Silicone Composites

OPH-silica (3.0–4.6 g) was suspended in a solution of ST-PDMS (3.8–5.0 g of 0.1% functional in 4–6 mL of pentane), this blended with a mixture of PDES (0.6–1.5 g), PAPS (0.4–1.2 g), PHMS (0.6–1.5 g), TO (30–110 mg), and pentane (2–6 mL), and the mix poured into an aluminum foil mold. Curing under ambient conditions for 6–18 h afforded a rigid porous foam, with a bulk density of 0.34–0.51 g mL^{-1}, pore volume of approx. 50–65%, and pore size of 0.1–6 mm. This was crushed, then granulated (Retsch ZM100 Centrifugal Mill) to afford a 0.3–5 mm particulate, which was washed with cyclohexane, dried in air at room temperature, then hydrophilized as above.

OPH-Polyurethane Foam Biocatalysts

These were prepared using a modification of the described methodology (Havens and Rase, 1993; Le Jeune et al., 1997; Yang et al., 1995). A 2:1 w/w mixture of Hypol 3000/5000 (4–6 g, containing 0.2% w/w of DABCO) was warmed to 35°C and stirred vigorously with an overhead stirrer fitted with a high shear inclined mixing head operated at 2,000–3,000 rpm. A mixture of OPH stock (0.24–1.2 mL of 2.29 kU mL^{-1} or 4–6 mL of 22.9 kU mL^{-1}, in 0.1 M TRIS, pH 7.5, with 1 mM $CoCl_2$, made up 4–6 mL with buffer, 10°C), glycerol tris[poly(propyleneglycol)] (0.4–1 g) and a Tween/PVA solution (0.4 or 0.6 mL of 10% w/v of a 1:1 mixture of Tween 60/80, and 15 w/v PVA in buffer containing 5% v/v of methanol) was added over 15 sec and mixing continued for 30 sec. The emulsion was poured into an aluminum foil mold and the foam allowed to gel/cure at 5°C for 30 min. The foam was soaked in capping solution (50 mL of 5% w/v glucosamine in 0.1 M TRIS, pH 7.5, with 1 mM $CoCl_2$) at 5°C for 20 min, washed with buffer (3 × 50 mL), then dried in air at 5°C for 10 h to give a sponge (4.3–4.6 g), with a pore volume of 71–82%, pores of 20–600 μm, and a bulk density of 0.31–0.39 g mL^{-1}. Granulates (0.15–3 mm) were prepared by homogenizing 1 cm cubes of the foam in buffer, followed by sieving and drying. A supported material was produced by casting onto nitrile glove base, followed by capping, washing, and drying, then cutting with a tissue cutter to give a foam of approx. 520 μm thickness.

Storage Stabilities of Biocomposites

Immobilizates were stored dry (<20% humidity), under 100% humidity, and wet at 5°C and at 19–20°C for 6 months, and at 44–46°C for 22 days. For comparison, native OPH (lyophilizate of a solution with 10 mg mL^{-1} of OPH in 50 mM TRIS buffer, pH 7.5, containing 5 mM calcium acetate and 10 μM cobalt acetate) was stored dry, and in solution (0.2 mg mL^{-1} and 1 mg mL^{-1}). For wet storage, the materials (0.4–1 mm particles of monolith, 2–3 mm particles of foams, 5 mm squares of supported thick film) were submerged in phosphate buffer (50 mM, pH 7.5, containing 5% methanol). For the experiments at 100% humidity, the samples were placed in open vials arranged in sealed jars containing filter paper disks wetted with sufficient buffer to saturate the headspace, and these placed in a refrigerator or incubator. After storage, the remaining OPH activities were determined by paraoxon hydrolysis assays.

Aqueous Assays of OPH Activity with Paraoxon

OPH activity was assayed with an aqueous assay using 5 mM of paraoxon as substrate. Biocatalyst was suspended in buffer (25 mM TRIS, pH 7.5, containing 5 μM $CoCl_2$) and

Table I. Immobilization of OPH onto PHOMS and 3-isocyanatopropylsiloxy silica.

Adsorbate	Support functionality (mmol g^{-1})[a]	OPH applied (mg g^{-1})[b]	OPH bound (%)[c]	Relative activity (%)[d]	Immobilization efficiency (%)[e]	Catalytic load (kU g^{-1})[f]
OPH-Poly(hydroxymethylsiloxane) adsorbates						
A1	—	1	>99	83	83	4.0 ± 0.3
A2	—	5	99	84	83	19 ± 2
A3	—	10	97	87	84	38 ± 6
A4	—	15	93	92	86	59 ± 7
A5	—	25	87	83	72	82 ± 11
A6	—	100	64	77	49	224 ± 14
A7	—	250	53	71	38	427 ± 21
OPH-(3-isocyanatopropylsiloxy-silica) adsorbates						
A8	0.93	0.1	>99	90	90	0.41 ± 0.03
A9		1	>99	92	92	4.2 ± 0.2
A10		5	>99	90	90	21 ± 3
A11		10	>99	88	88	40 ± 5
A12		15	98	89	87	60 ± 3
A13		25	97	85	82	94 ± 6
A14		50	93	81	75	172 ± 11
A15		100	88	73	64	293 ± 18
A16		200	88	71	62	573 ± 88
A17		300	87	68	59	809 ± 113
A18	0.97	0.1	>99	88	88	0.40 ± 0.02
A19		1	>99	88	88	4.0 ± 0.3
A20		5	>99	87	87	20 ± 3
A21		10	99	86	85	39 ± 3
A22		15	99	83	82	56 ± 6
A23		25	99	78	77	88 ± 5
A24		50	95	69	66	158 ± 12
A25		100	91	69	63	287 ± 24
A26		200	90	66	59	539 ± 60
A27		300	89	64	57	780 ± 112
A28		400	71	61	43	786 ± 98
A29	1.37	5	>99	81	81	19 ± 2
A30		25	>99	83	83	95 ± 8
A31		50	99	88	87	199 ± 17
A32		100	93	88	82	375 ± 31
A33		200	92	77	71	645 ± 45
A34		300	90	70	63	859 ± 93
A35		400	81	62	50	907 ± 140
A36	1.88	5	>99	78	78	18 ± 2
A37		25	>99	81	81	93 ± 8
A38		50	99	82	81	186 ± 14
A39		100	97	86	83	381 ± 17
A40		200	94	81	76	692 ± 39
A41		300	90	68	61	835 ± 70
A42		400	84	53	45	825 ± 79

[a]mmol of isocyanate per gram of silica.
[b]Amount of OPH applied to the support.
[c]Fraction of OPH bound to support, after washing with buffer.
[d]Activity of bound OPH relative to that of soluble OPH.
[e](OPH bound) × (relative activity).
[f]OPH activity per gram of support.
Activities were determined in duplicate.

paraoxon (10 mM in 25 mM TRIS, pH 7.5, containing 10% v/v propanol and 5 μM $CoCl_2$) added to a concentration of 5 mM. The biocatalyst was used at an OPH activity equivalence of 0.5, 1.0, or 2.0 U mL^{-1}, and the total volume of the assay mixture was 1–100 mL. The suspension was vigorously stirred at 25°C, aliquots removed, quenched with methanol, and centrifuged (15,000*g*, 10 min), and 4-nitrophenol determined by UV-VIS or HPLC. The background hydrolysis rate in the absence of OPH was 0.11–0.15 μmol min^{-1}.

OP D toxification Using Continuous Liquid and Gas Phase Bioreactors

Reactors were constructed from ϕ 1 cm × or ϕ 1.5 cm jacketed glass columns fitted with Teflon® endpieces and

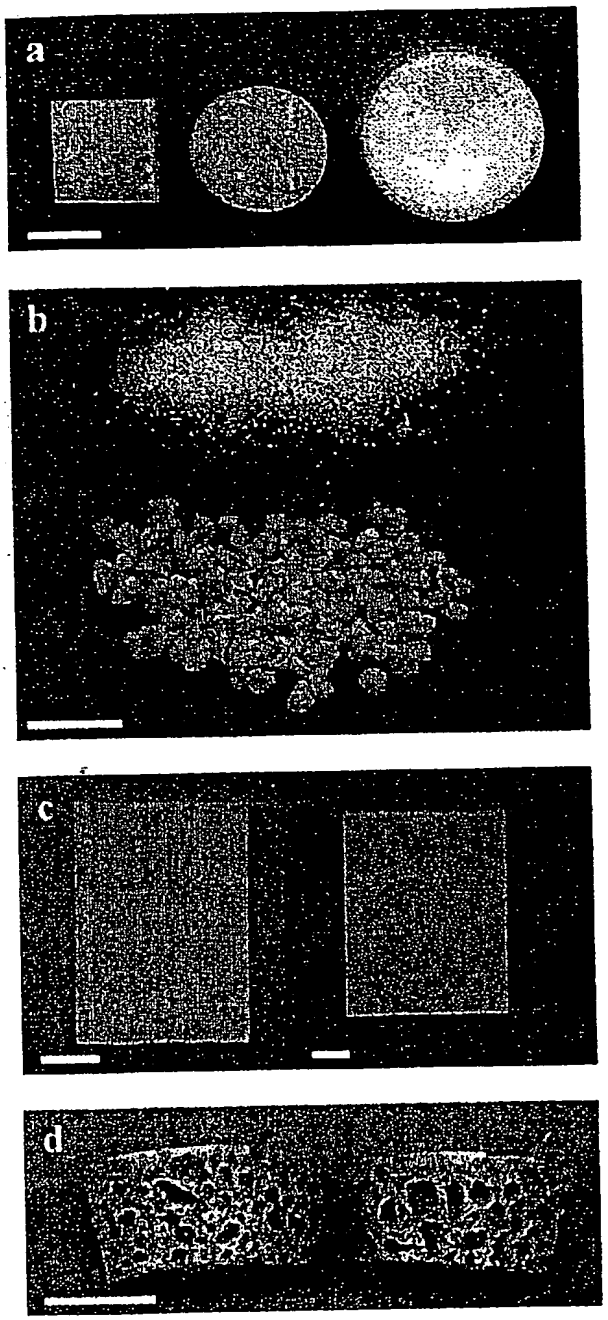


Figure 1. Representative examples of OPH-silica-silicone composite biocatalysts. a: Solid silicone monoliths: Left, 20% w/w OPH-silica-silicone; Middle, 20% w/w OPH-PHOMS in silicone; Right, 25% w/w OPH-silica in silicone. b: Solid granulates: Top, 300 μm granulate of 30% w/w sol-gel-immobilized OPH encapsulated in silicone; Bottom, 2–5 mm granulate of 25% w/w OPH-silica in silicone. c: Sheet and thick film composites: Left, 1 mm sheet of 20% w/w OPH-silica in silicone; Right, thick film of 15% w/w OPH-silica in silicone deposited on vinyl sheet. d: Foamed composite of 35% w/w OPH-silica in PHMS-blown silicone. Scale bars = 10 mm.

50 μm Teflon® frits (Omnifit) so as to give bed volumes of approx. 5, 10, 15, and 50 mL. The liquid phase reactors were fed with substrate solutions from glass reservoirs (placed in 0°C cooling baths) using syringe pumps (ORION) or peristaltic pumps (LabConco). The solutions were passed through 1/8 inch × 2 m long coils of Teflon® tubing immersed in a 30°C water bath just prior to the columns. With the gas phase reactor, a regulated air stream passing through two wash bottles containing buffer (25 mM TRIS, pH 7.5, containing 5 μM $CoCl_2$) and two ϕ 2.5 × 50 cm jacketed glass columns packed with dichlorvos-saturated Celite 640 pellets, provided the substrate feed. A second stream, passing through a buffer-containing wash bottle, was used as a make-up supply, to control the dichlorvos concentration at 9–10 μM. An empty ϕ 1 × 5 cm column fitted with a sampling/purge valve was attached to the outlet of the reactor column for sampling purposes. The columns were dry packed with biocatalyst, maintained at 30°C, and conditioned with substrate-free buffer or water-saturated air for 0.25–0.5 h prior to feeding paraoxon or dichlorvos. For paraoxon degradation by granular catalysts, OPH catalytic loads of approx. 112 U (0.04 mg OPH, silicone catalyst) and 89 U (0.045 mg OPH, polyurethane catalyst) were used for the reactors. In the case of the thick film catalysts, OPH loads of approx. 26 U (0.011 mg OPH, silicone catalyst) and 36 U (0.017 mg OPH, polyurethane catalyst) were employed for the reactors. For gas phase dichlorvos detoxification, OPH loads of approx. 1.78 kU (0.68 mg OPH, silicone catalyst) and 0.94 kU (0.48 mg OPH, polyurethane catalyst) were used for the reactors. In the case of DFP degradation, OPH loads of approx. 10.8 kU (5.0 mg OPH, silicone catalyst) and 13.8 kU (6.9 mg OPH, polyurethane catalyst) were used for the reactors. Liquid phase degradation of paraoxon and DFP were followed by UV spectrometry and HPLC, and by using a fluoride ion-sensitive electrode (Orion 290A ISE Meter), respectively. Detoxification of dichlorvos was monitored by GC. The background cleavage rates of paraoxon, dichlorvos, and DFP (for OPH-free composites) ranged over 0.12–0.17 μmol min^{-1}, 0.02–0.03 μmol min^{-1}, and 0.03–0.06 μmol min^{-1}, respectively.

RESULTS AND DISCUSSION

Adsorption of OPH onto Fumed Silica

Following our work on lipase immobilization onto the polymer poly(hydroxymethylsiloxane) (PHOMS) (Gill et al., 1999), we examined whether a similar approach could be utilized for accessing heterogeneous OPH biocatalysts. Soluble OPH was physically adsorbed onto PHOMS at applied protein/support ratios of 1–250 mg per gram (Table I, A1–A7), with the optimal specific activity of 92% and immobilization efficiency of 86% attained at an OPH load of 15 mg g^{-1}. Lower loads displayed near quantitative bindings but somewhat reduced specific activities, while higher loads suffered from lower OPH bindings and diminished

Table II. Encapsulation of OPH-PHOMS and OPH-silica in silicones.

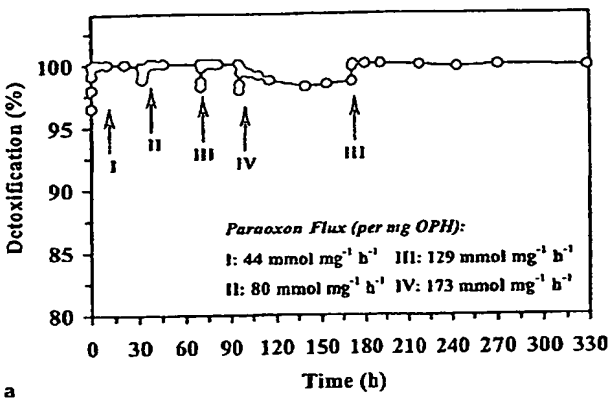
Silicone composite	Adsorbate used	Silicone biocatalyst composition (% w/w) Ad:PDMS:PDES:PAPS:PHMS	Activity loss (%)[a]	Relative activity (%)[b]	Catalytic load (kU g^{-1})[c]
Monolithic composites					
M2	A2	21:52:15:12:0	21	66	0.64 ± 0.08
M5	A5	30:45:15:10:0	19	67	20 ± 3
M6	A6	30:45:15:10:0	25	58	53 ± 4
M7	A7	30:45:15:10:0	28	51	93 ± 8
M18	A18	19:52:15:14:0	31	61	0.056 ± 0.004
M19	A19	17:53:15:14:0	23	68	0.52 ± 0.07
M20	A20	17:53:15:14:0	18	71	2.8 ± 0.2
M21	A21	17:53:15:14:0	26	64	4.9 ± 0.4
M22	A22	16:50:20:14:0	41	49	5.4 ± 0.3
M24	A24	16:50:24:10:0	32	47	17 ± 3
M25	A25	16:50:24:10:0	35	45	30 ± 4
M26	A26	16:50:24:10:0	32	45	58 ± 7
M28	A28	16:50:24:10:0	33	41	85 ± 5
M35	A35	38:35:22:5:0	15	53	313 ± 20
M42	A42	38:35:22:5:0	19	43	267 ± 17
Thick film composites					
T18	A18	12:63:20:5:0	41	52	0.028 ± 0.004
T19	A19	15:60:20:5:0	33	59	0.40 ± 0.05
T21	A21	15:60:17:10:0	28	62	4.3 ± 0.2
T23	A23	15:60:17:10:0	26	58	9.7 ± 0.8
T25	A25	15:60:20:5:0	23	53	33 ± 2
T28	A28	15:60:20:5:0	20	49	944 ± 73
Foamed composites					
F18	A18	30:44:8:10:3	24	67	0.089 ± 0.010
F22	A22	35:39:12:6:8	30	58	13 ± 2
F23	A23	35:39:12:6:8	28	56	22 ± 1
F25	A25	35:39:12:6:8	19	56	83 ± 11
F28	A28	35:39:12:6:8	15	52	229 ± 17

[a]Percentage of activity lost upon encapsulation.
[b]Activity of OPH-silicone relative to that of soluble OPH.
[c]OPH activity per gram of support. Encapsulations were quantitative, with no detectable loss of OPH during washing.
Activities were determined in duplicate or triplicate.
Abbreviations: Ad, adsorbate; PDMS, silanol-terminated poly(dimethylsiloxane); PDES, poly(diethyl silicate); PAPS, poly(3-aminopropylethoxysiloxane); PHMS, poly(hydrogenmethylsiloxane).

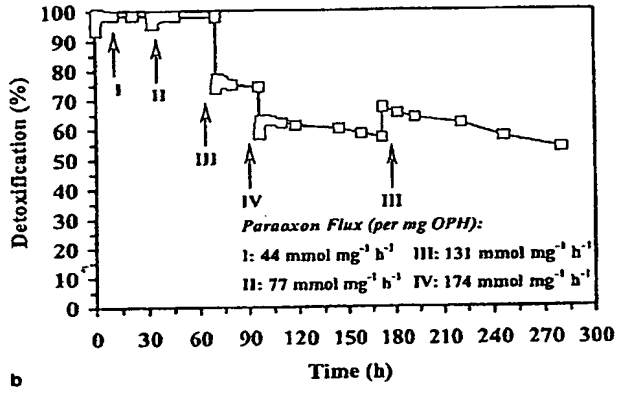
Table III. Immobilization of OPH onto polyurethanes.

Polyurethane foam	OPH applied (mg g^{-1})[a]	OPH bound (%)[b]	Relative activity (%)[c]	Immobilization efficiency (%)[d]	Catalytic load (kU g^{-1})[e]
P1	0.03	100	46	46	0.059 ± 0.008
P2	0.09	100	51	51	0.211 ± 0.031
P3	4.4	98	44	43	8.6 ± 0.9
P4	10	96	49	47	22 ± 2
P5	25	94	48	45	53 ± 4
P6	50	91	45	41	93 ± 4
P7	100	77	38	29	137 ± 16
P8	150	67	36	24	166 ± 28

[a]Amount of OPH applied onto support.
[b]Fraction of OPH bound to support, after washing with buffer.
[c]Activity of bound OPH relative to that of soluble OPH.
[d](OPH bound) × (relative activity).
[e]OPH activity per gram of support.
Activities were determined in duplicate.



a

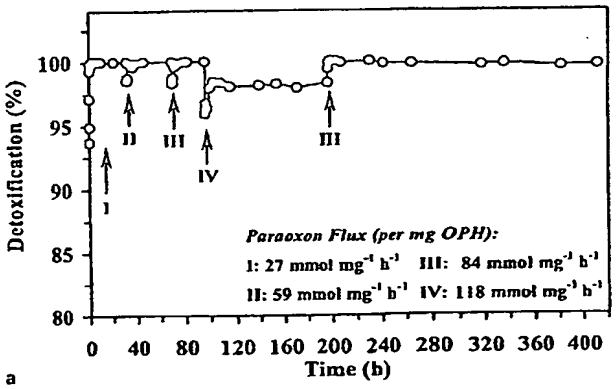


b

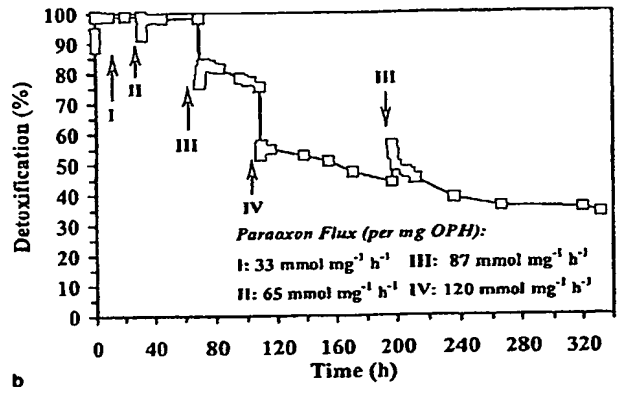
Figure 2. Paraoxon degradation by granular OPH-silicone and OPH-polyurethane. a: OPH-silicone M18 (2 g, approx. 112 U, total of 0.04 mg OPH) fed with 5 mM paraoxon at 5.9, 10.7, 17.4, and 23.3 mL min^{-1}. b: OPH-polyurethane P1 (1.5 g, approx. 89 U, total of 0.045 mg OPH) fed with 5 mM paraoxon at 6.1, 10.8, 18.3, and 24.3 mL min^{-1}. Both catalysts were ϕ 0.4–1 mm granulates, and were packed into ϕ 1 cm × 5 mL columns, fed with substrate dissolved in 25 mM TRIS, pH 7.5, containing 10% v/v isopropanol and 5 μM $CoCl_2$, 30°C. Swelling and some disintegration of the OPH-polyurethane was seen at 120 h.

specific activities. The maximal protein binding of 13.2% w/w occurred for OPH applied at above 240 mg per gram of support. Although the adsorbates appeared suitable for bulk catalyst applications, the reduced immobilization efficiencies at higher OPH loads were an issue for applications requiring high catalytic density materials.

Thus, we turned our attention to modified fumed silica as an alternative support to PHOMS. A 14 nm silica was modified via silylation with 3-isocyanatopropyltriethoxysilane, and OPH was covalently bound to this at applied loads of 0.1–400 mg g^{-1} (Table I, A8–A42). In contrast to PHOMS, almost quantitative binding of OPH was obtained up to loads of 100 mg g^{-1} with an appropriately functionalized silica (A36–A42), and respectable immobilization efficiencies of 43–63% were attained even at the maximal applied loads of 300 and 400 mg g^{-1}. Despite a doubling in the isocyanate functionality of the silica, no clear effect upon OPH-specific activity was discernable, although protein binding was considerably increased. It should be noted that, whereas binding of OPH to PHOMS is purely by physical adsorption, in the case of the derivatized silicas the protein is covalently anchored (urea and carbamate formation) via surface amino and hydroxyl groups. The specific activities of both types of immobilizate were higher than those reported for OPH immobilized by other methods (Andreopoulos et al., 1999; Caldwell and Raushel, 1991; Havens and Rase, 1993; Le Jeune et al., 1997), possibly reflecting milder binding processes, less constraining of bound OPH, and/or improved access of bound OPH. Indeed, the efficient



a



b

Figure 3. Paraoxon degradation by thick film OPH-silicone and OPH-polyurethane. a: OPH-silicone T18 (220–260 μm × 6 × 6 cm film, approx. 26 U, total of 0.011 mg OPH) fed with 5 mM paraoxon at 0.97, 2.11, 3.04, and 4.23 mL min^{-1}. b: OPH-polyurethane P1 (520 μm × 5 × 6 cm film, approx. 36 U, total of 0.017 mg OPH) fed with 5 mM paraoxon at 1.82, 3.65, 4.79, and 6.68 mL min^{-1}. Biocatalyst films were cast onto 0.2 mm nitrile sheet spiral packed into ϕ 1 cm × 5 mL columns, and fed with substrate dissolved in 25 mM TRIS, pH 7.5, containing 10% v/v isopropanol and 5 μM $CoCl_2$, 30°C. Swelling and loss of structural integrity was observed for the OPH-polyurethane at 160 h.

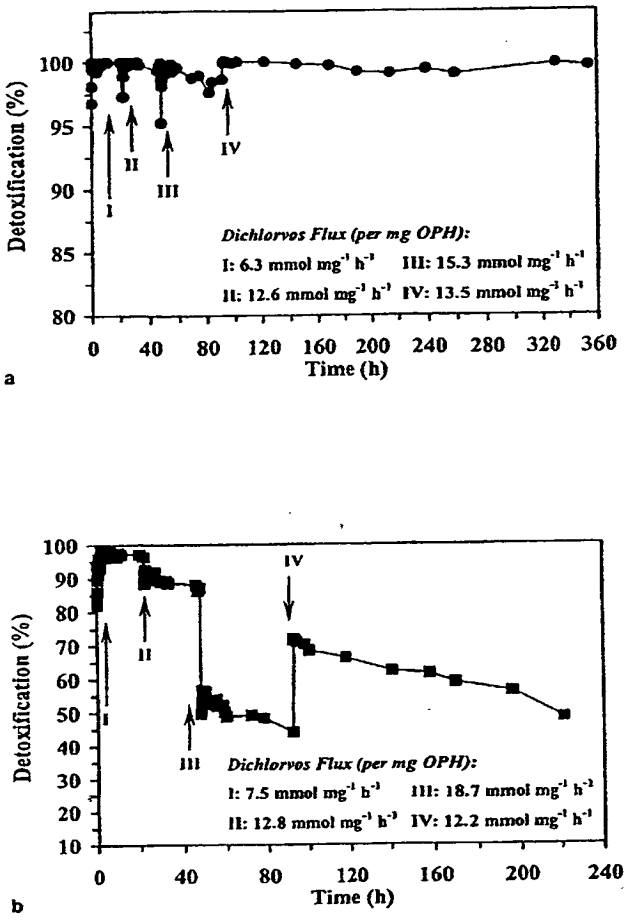


Figure 4. Gaseous dichlorvos degradation by OPH-silicone and OPH-polyurethane foams. a: OPH-silicone F18 (20 g, approx. 1.78 kU, total of 0.68 mg OPH) fed with 9.1–9.2 μM dichlorvos, at 7.81, 15.7, 19.1, and 16.9 L min^{-1}. b: OPH-polyurethane P1 (16 g, approx. 0.94 kU, total of 0.48 mg OPH) fed with 9.1–9.3 μM dichlorvos, at 6.05, 10.3, 15.1, and 9.8 L min^{-1}. The catalysts were ϕ 2–3 mm particulates, packed into ϕ 1.5 cm × 50 mL columns, fed with water vapor-saturated air containing substrate, 30°C. Swelling and some disintegration of the OPH-polyurethane polymer catalyst was observed at 130 h.

functioning of the OPH-silicas as compared with the OPH-PHOMSs, despite the covalent nature of the former, may reflect the importance of their nano-particulate nature.

Encapsulation of OPH-Silica into Reinforced Silicone Polymers

In the second step of immobilized OPH biocatalyst preparation, the OPH-PHOMSs and OPH-silicas were compounded with standard condensation-cure RTV silicone mixes (Kriecheldorf, 1996; Ziegler and Fearon, 1990) of silanol-terminated poly(dimethylsiloxane), and poly(diethyl silicate) and poly(3-aminopropylethoxysilane) crosslinkers, followed by tin(II)-mediated curing at room temperature, to form solid silicones which were readily granulated (Fig. 1a,b, Table II, M2–M7 and M18–M42). Up to 40% w/w of OPH-PHOMS or OPH-silica was incorporated into the silicones, with concomitant 18–41% reductions in OPH activity, resulting in overall specific activities of 41–71%. Part of this loss of OPH activity probably resulted from OPH denaturation by the silicone precursors and/or the ethanol released during curing. In addition, substantial mass transfer effects are expected to arise from the encapsulation of particles bearing surface-bound OPH within a bulk polymer matrix.

The functioning of OPH-PHOMSs and OPH-silicas both as dispersed catalyst phases as well as covalent, reinforcing structural components endows the composites with considerable physicochemical stability, and enables their manipulation as per conventional filled silicones (Kriecheldorf, 1996; Ziegler and Fearon, 1990). By varying the silicone composition, highly flexible and tear-resistant rubbers and tough rigid solids were formed (Fig. 1a–c), the former being readily applied to substrates such as nitrile gloving to produce highly active coatings (Fig. 1c, Table II, **T18–T28**) with promise as protective wear. Also, the use of poly(hydrogenmethylsiloxane) as a foaming agent furnished macroporous foams (Fig. 1d, Table II, **F18–F28**) which were suitable for treating gaseous substrates. The thick film and foamed immobilizates showed broadly comparable activity retentions to the monoliths, although increasing the adsorbate and/or PDMS content of the silicone mixes did appear to reduce activity losses during encapsulation.

The above results compared favorably with those obtained for OPH-polyurethanes (Caldwell and Raushel, 1991; Havens and Rase, 1993; Yang et al., 1995; Le Jeune et al., 1997). Thus, with in situ generated OPH-polyurethane foams we observed that although OPH bindings were comparable to the OPH-silica immobilizates, the specific activities and immobilization efficiencies were significantly lower (Table III, **P1–P8**). Even after encapsulation in silicone, the overall immobilization efficiencies obtained with the silicone composites were in the range of 10–65% higher than for the OPH-polyurethanes (Tables II, III). Also, the maximal OPH loads of the polyurethanes and silicone composites significantly differed at approx. 10% w/w and 14% w/w, respectively. It should be noted that no great attempt was made to optimize the OPH-polyurethane foam polymers used for comparison in these studies, and it is reasonable to expect that their activities and physicochemical attributes could be considerably improved via polymer engineering.

Liquid- and Gas-Phase Detoxification of Paraoxon, Dichlorvos, and DFP

The biocatalytic performances of representative composites were evaluated in comparison with the OPH-polyurethanes from the continuous detoxification of the OPs paraoxon and dichlorvos. As models for a clean-up catalyst and protective wear for aqueous nerve agent, a solid granulate and thick film on nitrile of OPH-silicone were compared with the

Table IV. Storage stabilities of OPH-silica, OPH-silicone, and OPH-polyurethane catalysts.

	Activity losses upon storage (% activity loss after 6 months at 5°C or 20°C, or 22 days at 45°C)								
	Dry			100% Humidity			Wet		
Biocatalyst	5°C	20°C	45°C	5°C	20°C	45°C	5°C	20°C	45°C
OPH-(3-isocyanatopropylsiloxy-silica) adsorbates									
A18	<5	<5	<5	<5	<5	12 ± 5	<5	<5	19 ± 6
A19	<5	<5	5 ± 2	<5	<5	16 ± 3	12 ± 3	16 ± 3	24 ± 8
A21	<5	<5	8 ± 2	11 ± 3	14 ± 6	15 ± 6	15 ± 4	19 ± 3	26 ± 7
A23	<5	6 ± 2	8 ± 3	16 ± 6	16 ± 3	15 ± 2	19 ± 5	24 ± 6	29 ± 9
A25	<5	6 ± 1	11 ± 2	13 ± 2	20 ± 3	18 ± 5	23 ± 7	31 ± 8	35 ± 14
<OPH-(3-isocyanatopropylsiloxy-silica)-silicone composites									
M18	<5	<5	<5	<5	<5	9 ± 1	<5	<5	9 ± 1
M19	<5	<5	6 ± 2	<5	<5	8 ± 2	<5	<5	12 ± 4
M21	<5	<5	9 ± 4	<5	<5	11 ± 4	<5	<5	9 ± 2
M23	<5	<5	<5	<5	<5	13 ± 1	<5	6 ± 1	11 ± 1
M25	<5	<5	8 ± 3	<5	6 ± 1	11 ± 6	<5	8 ± 1	12 ± 5
T23	<5	6 ± 2	6 ± 5	<5	7 ± 3	14 ± 2	7 ± 2	9 ± 3	13 ± 3
T25	<5	6 ± 1	9 ± 3	6 ± 2	7 ± 2	12 ± 3	9 ± 3	9 ± 2	16 ± 4
F23	<5	<5	8 ± 3	<5	<5	9 ± 2	<5	7 ± 2	11 ± 2
F25	<5	<5	9 ± 2	6 ± 1	8 ± 1	10 ± 4	6 ± 2	9 ± 2	13 ± 1
<OPH-Polyurethane foams									
P2	<5	<5	13 ± 3	<5	16 ± 3	25 ± 3	<5	20 ± 6	40 ± 6
P4	<5	7 ± 1	18 ± 5	<5	15 ± 1	32 ± 5	<5	22 ± 5	47 ± 8
P6	<5	11 ± 3	18 ± 2	7 ± 2	22 ± 3	40 ± 9	13 ± 2	30 ± 7	53 ± 8
P7	<5	13 ± 2	22 ± 6	12 ± 1	26 ± 2	43 ± 5	16 ± 3	33 ± 2	61 ± 12

The percentage of activity lost by the immobilizate during storage is reported. Activities were measured in duplicate or triplicate.

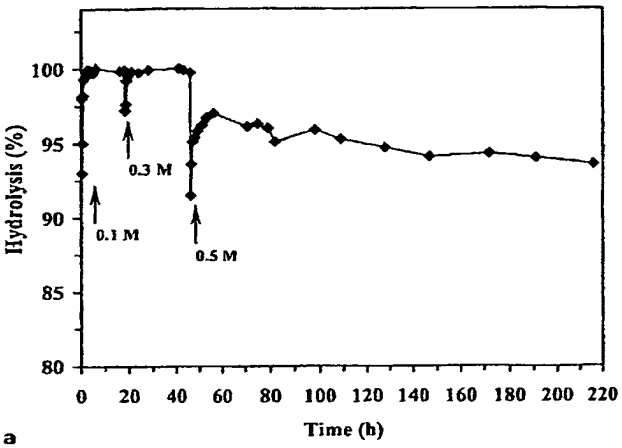
corresponding particulate and supported OPH-polyurethanes (Figs. 2, 3, respectively). Similarly, as a model for a personal filter against gaseous nerve agents a macroporous OPH-silicone foam was evaluated against an OPH-polyurethane in the detoxification of gaseous dichlorvos (Fig. 4). The OPH-silicone and OPH-polyurethane reactors were operated with similar fluxes (mmol of OP per mg of OPH per h), so as to allow the direct comparison of their detoxification profiles. The initial flows were selected to allow rapid conditioning of the catalyst and initial operation at >99% hydrolysis. Once stable conversions were attained the flows were increased until the detoxification capacities of the catalysts were exceeded and the operational stabilities were monitored thereafter.

Good operational profiles were observed for the OPH-silicone catalysts, with 100% detoxification being sustained over more than 200 h at organophosphate feed rates of 129, 84, and 15 mmol h^{-1} per mg OPH, respectively. These compared well with the significantly lower detoxification throughputs of 77, 65, and 8 mmol h^{-1} per mg OPH observed for the OPH-polyurethanes. For the OPH-silicones, the results imply a 1 L reactor degrading 1.56 mol (0.43 kg) of paraoxon per h, 1 m^2 of protective material deactivating 46 mmol (13 g) of paraoxon per h, and 100 cm^3 of gas filter detoxifying 36 mmol (7.9 g) of dichlorvos per h, with respective operational half-lives of about 8.8, 7.9, and 7.1 months. Higher OP fluxes could be accommodated by utilizing composites with higher OPH loads. In comparison, the profiles of the OPH-polyurethanes suggested throughputs of 0.52 mol (0.14 kg) of paraoxon per h for a 1 L reactor, 0.36 mmol (10 g) of paraoxon per h for 1 m^2 of protective wear, and 17.2 mmol (3.8 g) of dichlorvos per h for 100 cm^3 of gas filter, with corresponding half-lives of approx. 1.1, 0.6, and 0.4 months.

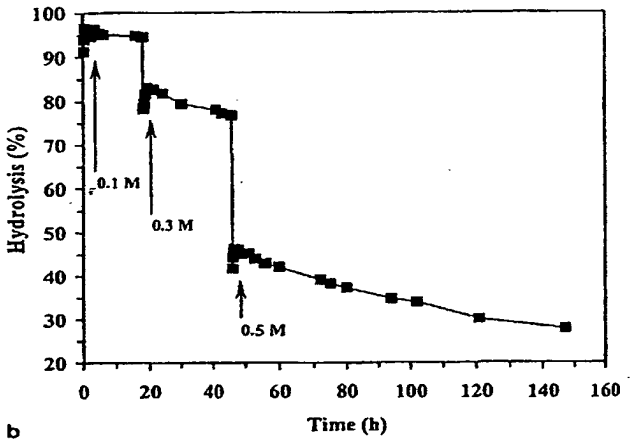
It is important to note that since different OPH catalytic loads were utilized for the various reactors, the above performance figures are based on the total amounts of OPH present in the catalysts in order to correct for this variation. These absolute figures take into account both the observed differences in the initial catalytic efficiencies (attributable to differences in OPH specific activity retentions during immobilization) as well as in the deterioration of the hydrolysis profiles during reactor operation (ascribed to differences in catalyst stability). Thus, the overall superiority of the silicone catalysts, as judged by these figures, reflects their higher immobilization efficiencies (Tables I and II vs. Table III) combined with improved stability as compared with the polyurethane foams.

Differences in the physical stability were also observed between the OPH-silicones and OPH-polyurethanes. Whereas the silicones were flexible or rigid solids or foams with considerable resistance to tear, abrasion, and compression, the polyurethanes were compressible semirigid foams with a relatively low fracture resistance. The former swelled slightly (5–10%) but retained their integrity upon prolonged immersion in the liquid feeds, while substantial swelling (30–40%) and disintegration of the polyurethanes was observed after 120–160 h.

The results indicate that OPH-silicones are superior biocatalysts, in terms of activity and operational stability, and



a



b

Figure 5. Degradation of DFP in an aqueous-organic feed by OPH-silicone and OPH-polyurethane. a: OPH-silicone M22 (2 g, approx. 10.8 kU, total of 5.0 mg OPH) fed with substrate solution at 0.51–0.53 mL min^{-1}. b: OPH-polyurethane P3 (1.6 g, approx. 13.8 kU, total of 6.9 mg OPH) fed at 0.72–0.75 mL min^{-1}. The catalysts were ϕ 0.4–1 mm granulates, packed into ϕ 1 cm × 5 mL columns, fed with 0.1 M, 0.3 M, or 0.5 M DFP (in a 1:2:3 isopropanol-ethanol-water, containing 0.15, 0.4, or 0.60 M triethanolamine, pH 7.5, and 20 μM $CoCl_2$), 30°C. Swelling and significant disintegration of the OPH-polyurethane was observed at 60 h.

suggest the possibility of employing these biocomposites for fabricating protective clothing and constructing compact bioreactors.

Having established the operation of the model OPH-silicone catalysts, we turned our attention to storage stability, which a critical concern for protective gear exposed to a variety of temperature/humidity conditions. The OPH-silicones lost less than 10% of activity during storage at 5°C or 20°C for 6 months, compared with the loss of up to 33% of activity for the OPH-polyurethanes (Table IV), and the observed half-lives of 14.9 days and 2.7 days for native OPH under the same conditions. At 45°C, the silicones and polyurethanes exhibited half-lives of 3.8–5.5 months and 0.6–2.8 months, respectively, comparing well with the reported stabilities of OPH-polyurethanes (Le Jeune et al., 1997). The stability differences between the silicone and polyurethane materials may reflect the greater susceptibility of surface-bound OPH to denaturing effects and its relative protection from these by enclosure in a silicone matrix. There were no significant differences in OPH activity losses between the monolithic, thick film and foamed silicones. However, the precursor OPH-silica immobilizates were considerably more prone to deterioration during wet storage (Table IV). This derived partly from the susceptibility of the OPH-silicas to leaching—up to 11% of OPH was lost, whereas there was no detectable leaching of enzyme from the silicones composites. The additional stability of the OPH-silicones may also reflect the inhibition of unfavorable conformational/unfolding transitions of OPH by the surrounding polymer framework.

For large-scale OP destruction, the low water solubility of most nerve agents would necessitate the use of mixed aqueous-organic feeds to deliver elevated OP concentrations for high-throughput processing. Hence, the functioning of a high catalytic density granular OPH-silicone catalyst was compared with that of an OPH-polyurethane in the hydrolysis of the G-series nerve agent model, diisopropyl fluorophosphate (DFP) in propanol-ethanol-buffer (Fig. 5). The catalysts displayed similar declines in activity with DFP as substrate as for the native enzyme—free OPH had an activity of 282 U mg^{-1} towards DFP, some 16.2-fold lower than for paraoxon, and the OPH-silicone and OPH-polyurethane had respective activities of 371 U g^{-1} and 546 U g^{-1} with DFP, and 5.39 kU g^{-1} and 8.63 kU g^{-1} (14.5- and 15.8-fold higher) with paraoxon. The OPH-silicone performed better than native OPH or OPH-polyurethane in the aqueous-organic medium—while the activity of native OPH in 1:2:3 propanol-methanol-buffer was 17–18% of that in pure buffer, the OPH-silicone held 72–75% of aqueous activity in this medium, compared to the 53–55% of activity retained by the OPH-polyurethane.

Despite the lower specificity of OPH toward DFP, and the decline in activity in the presence of organic cosolvents, the OPH-silicone efficiently degraded solutions containing up to 0.5 M DFP (Fig. 4a). These results correspond to a 1 L OPH-silicone loaded bioreactor detoxifying 3.34 mol (0.62 kg) of DFP per hour, with an estimated half-life of 4.7 months. In contrast, the performance of the OPH-polyurethane dropped considerably upon increasing the feed concentration from 0.1 M to 0.3 M (Fig. 4b), implying a detoxification capacity of about 2.07 mol (0.38 kg) of DFP per h, with a half-life of some 0.3 months. Also, the OPH-polyurethane swelled, then partially disintegrated during reactor operation, while the OPH-silicone retained full physical integrity throughout. These results suggest that OPH-silicones are superior immobilized catalysts for detoxifying

aqueous-organic feeds carrying high levels of OPs, and that they could be practical for high-throughput processing.

CONCLUSION

These results demonstrate that efficient OPH-silicone biocatalysts can be produced for detoxification of OPs. Although issues such as bulk fabrication and performance against weapons-grade nerve agents such as sarin and VX remain to be addressed, the facility of the technique, and the superior performance of the biocomposites with gaseous and liquid feeds makes this a promising avenue for immobilizing OP-degrading enzymes. Also, the high biocompatibility of silicones and their good physicochemical resistance makes OPH-silicones attractive for protective wear.

With the discovery of new OP-degrading enzymes, the development of engineered OPHs, and the production of recombinant microbes with cell-surface anchored OPHs (Grimsley et al., 1997; Richins et al., 1997; Watkins et al., 1997), one can envisage nerve agent destruction strategies based on biocatalytic silicone, polyurethane (Caldwell and Raushel, 1991; Havens and Rase, 1993; Yang et al., 1995; Le Jeune et al., 1997), and hybrid polymers, as well as fire-fighting foams (Le Jeune et al., 1998; Le Jeune and Russell, 1999).

The authors thank Drs. B. Brooker and R. Stenning (IFR, Reading, UK) for the use of microscopy facilities, and Dr. R. Valivety (Roche Vitamins, Inc.) for assistance during the preparation of the manuscript.

References

Andreopoulos FM, Roberts, MJ, Bentley MD, Milton Harris J, Beckman EJ, Russell AJ. 1999. Photoimmobilization of organophosphorous hydrolase within a PEG-based hydrogel. Biotechnol Bioeng 65:579–588.

Benning MM, Kuo JM, Raushel FM, Holden HM. 1994. Three-dimensional structure of phosphotriesterase: an enzyme capable of detoxifying organophosphate nerve agents. Biochemistry 33: 15001–15007.

Brin J. 1993. Ending the scourge of chemical weapons. Technol Rev April: 33–40.

Caldwell SR, Raushel FM. 1991. Detoxification of organophosphate pesticides using a nylon based immobilized phosphotriesterase from *Pseudomonas diminuta*. Appl Biochem Biotechnol 31:59–73.

Dumas DP, Caldwell SR, Wild JR, Raushel FM. 1989. Purification and properties of the phosphotriesterase from *Pseudomonas diminuta*. J Biol Chem 33:19659–19665.

Gill I, Ballesteros A. 1998. Encapsulation of biologicals within silicate, siloxane, and hybrid sol-gel polymers: an efficient and generic approach. J Am Chem Soc 120:8587–8598.

Gill I, Pastor E, Ballesteros A. 1999. Lipase-silicone biocomposites: efficient and versatile immobilized biocatalysts. J Am Chem Soc 121: 9487–9496.

Grimsley JK, Rastogi VK, Wild JR. 1997. Biological systems for the detoxification of organophosphorous neurotoxins. In: Sikdar SK, Irvine RL, editors. Bioremediation, principles and practice, vol 2. Biodegradation technology developments. Irvine, CA: Technomic. p 1–32.

Havens PL, Rase HF. 1993. Reusable immobilized enzyme/polyurethane sponge for removal and detoxification of localized organophosphate pesticide spills. Ind Eng Chem Res 32:2254–2258.

Holm FW. 1996. Scientific advances in alternative demilitarization technologies. NATO ASI Series, Disarmament Technologies, vol 6. Berlin: Kluwer Academic.

Hong SB, Raushel FM. 1996. Metal-substrate interactions facilitate the catalytic activity of the bacterial phosphotriesterase. Biochemistry 35: 10904–10912.

Hoskin FCG, Walker JE, Dettbarn WD, Wild JR. 1997. Hydrolysis of tetriso by an enzyme derived from *Pseudomonas diminuta* as a model for the detoxification of *O*-ethyl S-(2-diisopropylaminoethyl)methylphosphonothiolate (VX). Biochem Pharm 49:711–715.

Kolakowski JE, DeFrank JJ, Harvey SP, Szafraniec LA, Beaudry WT, Lai K, Wild JR. 1997. Enzymatic hydrolysis of the chemical warfare agent VX and its neurotoxic analogues by organophosphorous hydrolase. Biocatal Biotransform 15:297–312.

Kriecheldorf HR. 1996. Silicon in polymer synthesis. New York: Springer-Verlag.

Lai K, Stolowich NJ, Wild JR. 1995. Characterization of P-S bond hydrolysis in organophosphorothioate pesticides by organophosphorous hydrolase. Arch Biochem Biophys 318:59–64.

Landis WG, DeFrank JJ. 1991. Enzymatic hydrolysis of toxic organofluorophosphate compounds. In: Kameley D, Chakrabarty A, Omenn GS, editors. Biotechnology and biodegradation: methods and applications in biodegradation. Houston: Gulf Publishing. p 183–201.

Le Jeune KE, Russell AJ. 1999. Biocatalytic nerve agent detoxification in fire fighting foams. Biotechnol Bioeng 62:659–665.

Le Jeune KE, Mesiano AJ, Bower SB, Grimsley JK, Wild JR, Russell AJ. 1997. Dramatically stabilized phosphotriesterase-polymers for nerve agent degradation. Biotechnol Bioeng 54:105–113.

Le Jeune KE, Wild JR, Russell AJ. 1998. Nerve agents degraded by enzymatic foams. Nature 395:27–28.

Minton NA, Murray VSG. 1988. A review of organophosphate poisoning. Med Toxicol 3:350–375.

NRC (National Research Council) Reports. 1993. Alternative technologies for the destruction of chemical agents and munitions. Washington DC: National Research Council.

Rastogi VK, DeFrank JJ, Cheng TC, Wild JR. 1997. Enzymatic hydrolysis of Russian-VX by organophosphorous hydrolase. Biochem Biophys Res Commun 241:294–296.

Richins R, Kaneva I, Mulchandani A, Chen W. 1997. Biodegradation of organophosphorous pesticides by surface-expressed organophosphorous hydrolase. Nat Biotechnol 15:984–987.

Watkins LM, Mahoney HJ, McCulloch JK, Raushel FM. 1997. Augmented hydrolysis of diisopropyl fluorophosphate in engineered mutants of phosphotriesterase. J Biol Chem 272:25596–25601.

Yang F, Wild JR, Russell AJ. 1995. Non-aqueous biocatalytic degradation of a nerve-gas mimic. Biotechnol Prog 11:471–474.

Ziegler M, Fearon FWG. 1990. Silicon-based polymer science: a comprehensive resource, advances in chemistry series, 224. Washington DC: American Chemical Society.